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Aggregation pheromones in Collembola (Apterygota); a biotic cause of aggregation

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INTRODUCTION

Many field studies (e.g. those of MILNE, 1962; USHER, 1969 and JOOSSE, 1970) have shown that Collembola have an aggregated distribution pattern. Correlations have been estimated in the field between various environmental factors and the density of Collembola (e.g. POOLE, 1962 and VERHOEF, 1977).

In the few experimental studies, performed to analyse causes of aggregation, the environmental factors: relative humidity, moisture, food and soil type appeared to be important (CHRISTIANSEN, 1970; JOOSSE, 1971; JOOSSE & VERHOEF, 1974; BARRA & CHRISTIANSEN, 1975 and USHER & HIDER, 1975).

High degrees of aggregation are also found however in relatively uniform habitats (a.o. MILNE, 1962), as well as in experimentally homogeneous conditions (VERHOEF, in preparation). This points to « some kind of mutual attraction » (USHER & HIDER, 1975).

The aim of this study is to investigate the possible existence of chemical stimuli playing a rôle in the formation of aggregations in Collembola.

MATERIALS AND METHODS

The collembolan species used for this study were *Tomocerus minor* (Lubbock) and *Orchesella cincta* (L.), collected in the surface litter layer of a mixed birch-pine forest near Hilversum and *Orchesella villosa* (Geoffroy), collected in a pine plantation in the Flevopolder (both located in the Netherlands).

Preference tests were carried out in PVC jars (15 cm ∅) with a plaster of Paris floor of 1,5 cm, which was kept damp with distilled water. Small cages,

fitting in one of the nine equal sectors of the box, were used to enclose 100 individuals in order to « condition » that part of the floor. After 20 hours, cages and animals were removed, a fixed number of fresh test animals was introduced into the box and the influence of the conditioned place upon the resultant distribution was determined.

To exclude visual stimuli, these tests were performed in complete darkness. Observations were made using photographs taken by flash-light.

Since Collembola have an aggregated distribution in an environment without previously conditioned places, preference for the conditioned sector was determined by the binomial test on the frequency of the highest number of animals present in the conditioned sector.

RESULTS AND CONCLUSIONS

1. Aggregation on conditioned places.

The preference tests have shown clearly that each of the 3 investigated species prefers significantly the sector conditioned by its own species to non-conditioned sectors.

When conditioning took place by one of the sexes, preference tests for the same and the opposite sex made clear that there is no difference in production and perception between the two sexes.

In those preference tests in which the conditioning species and the tested species were different, it appeared that both species of *Orchesella* do not show any reaction on each other (: species specificity) or on *Tomocerus minor*.

Tomocerus minor however, shows a positive response to both species of the genus *Orchesella* (: absence of genus specificity).

A choice test for 1 species between 4 different possibilities: 3 sectors, each conditioned with a different species, and the 6 control sectors, made clear that each species can discriminate between conspecific and non-conspecific conditioning. The reaction of *Tomocerus minor* however, is not strictly species specific.

These results give rise to the conclusion that these Collembola use pheromones in the formation of aggregations.

2. Location of the organ(s) of pheromone perception.

Since the olfactory sense in insects is mainly a function of the antennae (PAYNE, 1974), the antennae of *Orchesella cincta* were excised to identify their rôle in the pheromone perception. After anaesthesia with ether, the antennae were amputated between the first and the second segment. The ether treatment did not affect reference animals in their behaviour. The operated ones were capable of all kinds of activities, like feeding, reproduction and reaction on environmental factors.

In none of the preference tests the conditioned sector was preferred. The test animals did not form any aggregation. In light conditions, however, these animals were able to aggregate.

These results show that antennae play an important rôle in the pheromone perception.

3. **Mechanisms for aggregating on the conditioned place.**

Movement towards the source and/or cessation of locomotion on the insect's arrival at the source, are generally considered to be the behavioural responses to a chemical source (SHOREY, 1973). Both, distant attraction and inhibition of locomotion were investigated.

a. *Locomotory activity at a short distance of the conditioned place.*

The active space (WILSON, 1970) of the pheromone was investigated over the, relatively, short distance of 6 mm. For that aim, small boxes with gauze floors were placed alternately on conditioned and non-conditioned parts of a plaster floor, with the gauze floor 6 mm above the plaster floor.

The locomotory activity of individuals of *Orchesella cincta* above the conditioned places appeared to be significantly lower than that above the non-conditioned ones.

This distant action of the pheromone implicates that the pheromone (or a component) is olfactorially active.

b. *Locomotory activity on the conditioned place.*

The same procedure as in *a.* was used, with the only exception that the boxes with a gauze floor were substituted by rings, so that contact was allowed.

The activity on the conditioned places appeared to be significantly ($P < 0.001$) lower than that on the non-conditioned places.

DISCUSSION

Although primitive insects are considered to have no pheromones at their disposal (MOORE, 1967), the present study, together with a report on a sex pheromone in *Sinella curviseta* Brook (WALDORF, 1974), gives evidence of the existence of pheromones in Collembola.

It has been shown that places conditioned by these animals may be considered as pheromone sources, causing aggregation. Although perception is not strictly species specific, all 3 investigated species appear to be more responsive to their own pheromones, than to those produced by other species. Both males and females appear to produce and perceive the aggregation pheromone.

The distant effect of the pheromone and the perceptive rôle of the antennae point to an olfactorially acting pheromone.

The behavioural reaction of the animals is probably orthokinetic: reduction in locomotory activity occurs as the pheromone concentration increases. According to DETHIER *et al.* (1960) this pheromone is called an arrestant.

In the investigation of causes of aggregation in Collembola, this is the first evidence of the existence of mutual attraction by means of chemical stimuli.

In higher insects this appears to be widespread (BLUM, 1974). Generally, the aggregation pheromones are olfactorially active, as in Collembola, though compa-

red with these, their active space may be wider. In some insects chemotactic orientation to distant pheromone sources has been proved (BELL *et al.*, 1973).

Concerning the significance of aggregation in Collembola, the very existence of pheromones inducing aggregation emphasizes its importance. JOOSSE (1970) found protection against desiccation as a function of aggregation caused by and existing in optimal moisture conditions. It is quite probable that aggregation pheromones and environmental factors cooperate in the formation of aggregations on suitable places (VERHOEF, in preparation). JOOSSE (1970) also stressed the importance of aggregation as a meeting system in those species where reproductive behaviour is not yet developed. As mating in Collembola is indirect, involving deposition of spermatophores by males and subsequent taking up by females (SCHALLER, 1971), aggregation of both sexes may enhance the efficiency of reproduction.

Once aggregated, primer pheromones may elicit reproductive synchrony, a phenomenon common in insect species possessing aggregative propensities (BLUM, 1974 and WALDORF, 1974).

Further investigation concerning pheromones in Collembola is in progress; a more extensive paper will be published elsewhere.

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